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Foaling-management practices associated with the occurrence of enterocolitis attributed to *Clostridium perfringens* infection in the equine neonate

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Abstract

Enterocolitis associated with *Clostridium perfringens* (*C. perfringens*) infection in neonatal foals is often severe and has been associated with a high case-mortality risk. We designed a premises-based survey to evaluate the associations of regional foaling practices, premises environmental management, periparturient foal and brood-mare management, and periparturient brood-mare ration with the occurrence of neonatal enterocolitis attributed to *C. perfringens* infection. Potential risk factors individually associated with enterocolitis were breed type, housing type at foaling and in the first three days of life, ground/floor surface type at foaling and in the first three days of life, brood-mare ration before and after foaling, and the presence of livestock other than horses on the premises in the past. From the multivariable-logistic regression models, six variables were significantly associated with an increased risk of the outcome of interest ($p<0.05$): foals of the stock horse type, housing in a stall or drylot in the first three days of life, other livestock present on the premises in the past, foal born on dirt, sand or gravel surface, and low amounts of grass hay and grain fed post-partum. Low grain amounts fed pre-partum represented a decreased risk of the outcome of interest.

Keywords: *Clostridium perfringens*; Horse; Enterocolitis; Foaling management

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1. Introduction

Enterocolitis associated with *Clostridium perfringens* (*C. perfringens*) infection in neonatal foals is often severe and has been associated with a high case-mortality risk (Niilo and Chalmers, 1982; Sims et al., 1985; Howard-Martin et al., 1986; Pearson et al., 1986; Dart et al., 1988; Drolet et al., 1990; Bueschel et al., 1998; East et al., 1998). A 10-year retrospective study performed at Colorado State University Veterinary Teaching Hospital (CSU-VTH) showed an overall case-mortality risk of 54% (East et al., 1998). In 1997, the case-mortality risk in this study was 100% if *C. perfringens* type C alone was isolated from anaerobic fecal or intestinal cultures of affected foals (East et al., 1998). Because this disease had a high case-mortality risk despite medical treatment, we wanted to determine risk factors for the disease. We therefore designed a premises-based survey to evaluate the association of regional foaling practices, premises environmental-management practices, periparturient foal- and brood-mare-management practices, and periparturient brood-mare ration with the occurrence of neonatal enterocolitis attributed to *C. perfringens* infection. The objective of this study was to evaluate factors that might form the basis for intervention strategies and to suggest avenues for future research.

2. Materials and methods

Study population. A search of the medical-records database for 1996 and 1997 was used to identify all clients with foals examined at CSU-VTH at <10 days of age. This included foals accompanying their dams (i.e. the dam was the primary patient) as well as foals examined/hospitalized as the patient (i.e. *C. perfringens* enterocolitis, colic, non-clostridial diarrhea, sepsis, prematurity, orthopedic disease, neurologic disease, umbilical complications, etc.). One hundred and twenty-five foals were identified from 119 different premises. Of these premises, 23 (10 in 1996 and 13 in 1997) had at least one foal diagnosed at CSU-VTH with enterocolitis attributed to *C. perfringens* infection.

Each premises was randomly assigned a number to provide client-response anonymity. The premises' survey number was linked to the premises' zip code to allow tracking of spatial demographics or regional grouping of survey responses. These procedures were performed to comply with the CSU human-research council's requirements (i.e. no personal premises identifiers could be associated with the survey questionnaire responses).

The outcome of interest for this study was the occurrence in foals of clinical disease compatible with a diagnosis of enterocolitis attributed to *C. perfringens* infection. The case definition was any foal with signs of colic, diarrhea, or sudden death that had a positive anaerobic fecal or intestinal culture for *C. perfringens* or any foal with clinical signs of both bloody diarrhea and colic at <10 days of age. This case definition was narrow and may have excluded some affected foals with less-specific clinical signs. Respondents were asked to report on all foalings occurring in 1996 and 1997 in which a live foal was born.

Survey design. A comprehensive client questionnaire[2] was developed with the approval of the CSU human-research council to collect information on foaling practices, foal management, environmental management, and brood-mare management including ration. Questions focused on the management of the brood-mare pre-partum and on the brood-mare and foal during the first 10 days post-partum, with an emphasis on the first three days post-partum (i.e. day 0 to day 3).

Each questionnaire was accompanied by extensive written instructions for each variable to minimize potential misinterpretation by the respondent and to maximize accuracy of responses. The questionnaire was pilot tested on a premises that indicated that the questions were understandable and required approximately 1 h to complete. A total of 42 variables were included in the questionnaire. For each variable, respondents were offered several choices of responses and they could also provide written comments explaining their response. The premises were given one and a half months to complete and return the questionnaire. No repeatability testing was possible due to the required maintenance of anonymity of respondents.

All respondents were provided with a telephone number to contact one of the investigators (LME) directly with questions or concerns regarding the survey. A stamped and self-addressed return envelope was included in each questionnaire packet. One week after mailing out the survey, a follow-up phone call was made to all questionnaire recipients to answer questions and improve response percentage. No follow-up on questionnaire responses was possible because questionnaires were not linked to a respondent name or address because of the regulatory requirements of the CSU human-research council. Thus, neither could differences between respondents and non-respondents be determined nor could clarification of responses on the returned questionnaires occur.

Data analysis. The data were captured electronically and checked to identify and correct data-entry errors. Each foal was categorized as a case or non-case based on the case definition. Each potential risk factor was screened for a significant association with the outcome status of the foal. Each categorical variable was evaluated using χ^2 or a Fisher's exact test (performed on variables where greater than 20% of the cells in the chi-square analysis had an expected count of <5) (Iman, 1994). Continuous variables related to amounts of feed offered to the brood-mares prior to and after foaling were evaluated using a Wilcoxon-rank sum test because of the small number of cases (Rosner, 1986).

In some cases, categories were collapsed due to sparse data or due to biologic compatibility of data. For data analysis, the multiple breed types were collapsed into two categories: stock-horse breeds (Quarter Horse, Appaloosa, and Paint) and all other breeds. The categories for ground/floor surface types were collapsed based on the ease of cleaning and disinfecting them. Ground/floor surfaces were categorized as either difficult to clean/disinfect (dirt, sand, or gravel) or more-easily cleaned/disinfected (cement, rubber, or wood). The ground/floor surface in pasture and drylots was classified as dirt, sand, or gravel. All housing variables referring to a stall or foaling stall were collapsed into one variable: stall. All stalled foals with turnout to a paddock or pasture were

[2] The survey questionnaire is available from Dr. L.M. East upon request.

combined into one variable: stall and outside access. No collapsing was performed on the housing variables referring to pasture or drylot alone.

Any variables that were associated with the outcome ($p<0.2$) by χ^2, a Fisher's exact test or the Wilcoxon-rank sum test and met the inclusion criteria ($\geq 5\%$ of the study population exposed), were eligible for inclusion in the multivariable logistic-regression model. Because the data regarding foal status were clustered by premises and, in some instances by mare, Sudaan (Shah et al., 1996) was used for the modeling (McDermott and Shukken, 1994; McDermott et al., 1994). All of the candidate variables were entered into the model. Variables were removed from the model until all remaining had a Wald statistic $p<0.05$. Sudaan does not report a design-based log-likelihood that can be used to compare models. Therefore, the variable with the largest Wald-statistic p-value was removed sequentially until all remaining variables had a Wald-statistic $p<0.05$. From the outcome of this model, odds ratios were determined for the remaining variables. The continuous feed variables were evaluated to determine if the risk of being a case was linear in the logit scale (Hosmer and Lemeshow, 1989a). In all cases, the risk was not linear in the logit, and the data suggested that a binary form for the variable would be the most appropriate. The dicotomous feed variables were constructed and were available for model building. Once a main-effects model was constructed, 2-way interaction terms were added to the model and evaluated based on the size of the Wald statistic p-value. Interactions of breed type by the amount of feed offered pre- and post-foaling were evaluated. A design-based goodness-of-fit statistic is not available using Sudaan. The Hosmer–Lemeshow (Hosmer and Lemeshow, 1989b) statistic for the final model, neglecting the clustered nature of the data, was calculated as an approximate assessment of the model fit.

3. Results

The response proportion for the survey was 46% (55/119 premises). More premises reported on foalings for 1997 (38%) compared to 1996 (28%). Twenty-three premises (23/55, 42%) provided data on foalings for both years. Overall, 17 of 55 premises were reported having one or more foals meeting the case definition. Most of the premises ($n=11$) with case foals had a single case. Five premises had two case foals and one premises had five case foals. Nine of the premises with one or more cases completed surveys for both years, whereas only 14 of the control premises completed surveys for both years. Seven of the premises had one or more cases in 1996 only, six of the premises had one or more cases in 1997 only, and four premises had cases in both years. Data were reported on 205 foals (75 foals in 1996 and 130 foals in 1997) from 174 different mares. Thirteen percent of the foals (26/205: 16 in 1996 and 10 in 1997) met the case definition. Two brood-mares had affected foals in both years covered by the study.

This survey is the first summary of the regional foaling practices; thus, the general frequency data obtained from the survey will be summarized to characterize the population studied (Table 1). Most foals (79.9%) were born in March ($n=39$), April ($n=70$), or May ($n=50$). The median number of foals born on each premises was 1 for 1996 and 2 for 1997 (range from 0 to 30 foals per premises in 1996 and 0 to 26 foals per

Table 1
Animal-level on premises demographics environmental and animal descriptive variables (Colorado, USA; 1996–1997)

Variable	Categories	Frequency in control animals (%)	Frequency in case animals (%)
Birth year (n=205)[a]	1996	59 (33)	16 (62)
	1997	120 (67)	10 (38)
Breed of brood-mare (n=203)[a]	Arabian, Morgan, and Gaited horse	36 (20)	2 (8)
	Mustang, Pinto, and Pony	5 (3)	0 (0)
	Quarter Horse, Paint, and Appaloosa	100 (56)	22 (84)
	Thoroughbred	24 (14)	2 (8)
	Warm blood and Draft	12 (7)	0 (0)
Sex of foal (n=202)	Female	90 (51)	14 (58)
	Male	88 (49)	10 (42)
Birth month of foal (n=199)	January, February, or March	41 (23)	8 (33)
	April, May, or June	126 (72)	15 (63)
	July, August, or September	8 (5)	1 (4)
Environmental climate at foaling (n=202)	Dry, cold	43 (24)	5 (21)
	Dry, warm	103 (58)	16 (67)
	Wet, cold	19 (11)	3 (12)
	Wet, warm	13 (7)	0 (0)
Premises facility type (n=202)	Boarding facility	6 (3)	2 (8)
	Breeding farm	63 (35)	4 (17)
	Private farm/ranch	101 (57)	16 (67)
	Training center	9 (5)	2 (8)
Foals dying post-partum (n=205)	Yes	8 (4)	17 (65)
	No	171 (96)	9 (35)
Number of previous foalings/mare (n=196)	0	33 (19)	7 (31)
	2–5	97 (56)	12 (52)
	>5	43 (25)	4 (17)
Housing type day 0 (n=201)[a]	Drylot	14 (8)	4 (15)
	Pasture	44 (25)	2 (8)
	Stall/foaling stall	83 (47)	15 (58)
	Stall/foaling stall with pasture	15 (9)	2 (8)
	Stall with run	19 (11)	3 (11)
Housing type day 3 (n=201)[a]	Drylot	15 (9)	3 (12)
	Pasture	40 (23)	1 (4)
	Stall/foaling stall	59 (34)	14 (54)
	Stall/foaling stall with pasture	36 (20)	4 (15)
	Stall with run	25 (14)	4 (15)
Ground/floor surface type day 0 (n=200)[a]	Dirt, sand, or gravel	122 (70)	25 (96)
	Cement, rubber, or wood	52 (30)	1 (4)
Ground/floor surface type day 3 (n=200)[a]	Dirt, sand, or gravel	126 (72)	23 (88)
	Cement, rubber, or wood	48 (28)	3 (12)

Table 1 (*Continued*)

Variable	Categories	Frequency in control animals (%)	Frequency in case animals (%)
Cleaned foaling area between foals (n=199)	Yes	121 (70)	17 (65)
	No	52 (30)	9 (35)
Other livestock on premises in past (n=199)[a]	Yes	85 (48)	16 (67)
	No	90 (52)	8 (33)
Other livestock on premises at present (n=203)	Yes	81 (46)	14 (54)
	No	96 (54)	12 (46)
Brood-mare management pre-partum			
Cleaned udder (n=198)	Yes	32 (18)	6 (25)
	No	142 (82)	18 (75)
Cleaned perineum (n=198)	Yes	38 (22)	6 (25)
	No	136 (78)	18 (75)
C. perfringens vaccination once (n=198)	Yes	5 (3)	7 (29)
	No	169 (97)	17 (71)
C. perfringens vaccination twice (n=198)	Yes	11 (6)	0 (0)
	No	163 (94)	24 (100)
Dewormed (n=198)	Yes	161 (93)	23 (96)
	No	13 (7)	1 (4)
Routinely vaccinated (n=200)[b]	Yes	171 (97)	22 (92)
	No	5 (3)	2 (8)
Wrapped tail (n=198)	Yes	49 (28)	4 (17)
	No	125 (72)	20 (83)
Brood-mare milk production (n=198)	Agalactic	3 (2)	0 (0)
	Light lactation	9 (5)	1 (4)
	Moderate lactation	67 (38)	9 (38)
	Heavy lactation	95 (55)	14 (58)
Brood-mare ration pre-partum			
Alfalfa hay (kg/day)[a]	0	75 (45)	8 (33)
	0.45–6.4	49 (29)	5 (21)
	6.8–11.4	24 (14)	11(46)
	>11.4	22 (13)	0 (0)
Grain (kg/day)[a]	0	37 (22)	10 (42)
	0.45–2.3	89 (52)	12 (50)
	2.7–4.5	35 (21)	2 (8)
	>4.5	9 (5)	0 (0)
Grass hay (kg/day)[a]	0	73 (43)	13 (54)
	0.45–6.4	11 (6)	5 (21)
	6.8–11.4	57 (34)	2 (8)
	>11.4	29 (17)	4 (17)
Brood-mare ration post-foaling			
Alfalfa hay (kg/day)[a]	0	77 (45)	7 (29)
	0.45–6.4	47 (28)	5 (21)
	6.8–11.4	30 (18)	12 (50)
	>11.4	16 (9)	0 (0)
Grain (kg/day)[a]	0	29 (17)	6 (25)
	0.45–2.3	85 (50)	16 (67)
	2.7–4.5	36 (21)	1 (4)
	>4.5	20 (12)	1 (4)

Table 1 (*Continued*)

Variable	Categories	Frequency in control animals (%)	Frequency in case animals (%)
Grass hay (kg/day)[a]	0	68 (40)	13 (54)
	0.45–6.4	15 (9)	5 (21)
	6.8–11.4	46 (27)	2 (8)
	>11.4	41 (24)	4 (17)
Foal treatments (n=200)			
C. perfringens C/D anti-toxin	Yes	8 (4)	1 (5)
	No	170 (96)	21 (95)
Prophylactic antimicrobials (n=200)			
Metronidazole	Yes	11 (6)	1 (4)
	No	167 (94)	21 (96)
Penicillin	Yes	21 (12)	4 (18)
	No	157 (88)	18 (82)
Trimethoprim-sulfamethoxazole	Yes	5 (3)	1 (5)
	No	173 (97)	21 (95)
Probiotics (n=205)	Yes	30 (17)	3 (12)
	No	149 (83)	23 (88)
Foal gastrointestinal disease at <10 days (n=205)			
Colic	Yes	5 (3)	0 (0)
	No	174 (97)	26 (100)
C. perfringens enterocolitis	Yes	0 (0)	26 (13)
	No		
Diarrhea (non-clostridial)	Yes	27 (15)	4 (15)
	No	152 (85)	22 (85)

[a]Potential risk factors associated with the occurrence of enterocolitis attributed to *C. perfringens* infection in the equine neonate, $p \leq 0.2$.

[b]Vaccination against influenza, equine herpes virus, tetanus, and equine eastern/western encephalitis.

premises in 1997). The environmental conditions at foaling were predominantly dry (83%) with warm (59%) or cold (24%) environmental temperatures. The most-common type of foaling facility was a private farm/ranch (58%) or a commercial breeding farm (33%) (Table 1). Most premises (99%) were located along the corridor of Interstate Highway 25 from Cheyenne, WY to Pueblo, CO. The average transport time to CSU-VTH hospital for respondents ranged from approximately 10 min to 5 h. Multiple breeds of mares were represented (Table 1). The remainder of the variables for environment, brood-mare management, foal management and feeding are summarized in Table 1.

Seventy-six percent of brood-mares received grain pre-partum and 82% of brood-mares received grain post-partum. For premises where grain was fed, the reported average daily amount of grain fed pre-partum was 1.8 kg with a range 0.45–7.2 kg and post-partum was 2.2 kg with a range 0.45–9.5 kg. Fifty-seven percent of mares were reportedly fed alfalfa hay pre-partum and 57% were fed alfalfa hay post-partum. For premises where alfalfa hay was fed, the reported average daily amount of alfalfa hay fed pre-partum was 7.2 kg with a range 0.9–18 kg and post-partum was 6.8 kg with a range 0.9–18 kg. Grass hay was reportedly fed to 66% of the mares pre-partum and 68% of mares post-partum. For premises where grass hay was fed, the reported average daily amount of grass hay fed pre-partum was 8.5 kg with a range 1.4–22.7 kg and post-partum was 9.4 kg with a range 0.9–27 kg. All of the ration amounts were the respondents' estimates of the amounts fed in pounds. Table 1 illustrates the percentage of case and control brood-mares with case and control foals fed each ration ingredient by weight in kilograms (kg) both before and after foaling.

Only premises that had case foals currently or in the past administered oral metronidazole. The spectrum of gastrointestinal diseases reported for all foals <10 days of age encompassed: non-bloody diarrhea, enteric disease attributed to *C. perfringens*, and colic. Five of the 179 (3%) non-case foals were reported to have had colic and 27 (15%) were reported to have had diarrhea. Twelve percent of the live-born foals (25/205) died by less than 10 days of age with the largest proportion of deaths attributed to *C. perfringens* infection (68%, 17/25, p=0.001).

The owners were also given the opportunity to provide written comments. Most comments came from respondents that had foals meeting the case definition. Some respondents reported that since they moved the foaling area from a calving shed, dairy barn, goat pen, or diarrheic calf's stall, they had no further problems with enterocolitis attributable to *C. perfringens* infection. Other respondents reported that instituting such prophylactic measures as vaccinating mares pre-partum with *C. perfringens* type C/D toxoid, propylactic administration of oral metronidazole, and/or propylactic administration of oral *C. perfringens* type C/D antitoxin to the neonatal foals had prevented subsequent disease.

Of the 41 variables examined, eight were eliminated from further analysis because >95% of the responses were in a single category (i.e. oral trimethoprim-sulfamethoxazole, oral *C. perfringens* type C/D antitoxin, oral *C. perfringens* type C/D hyperimmune plasma, other antimicrobials, sudden death, oral yogurt, routine mare vaccination, and other treatment interventions). Most of these nine variables were not associated with the outcome of interest (Fisher's exact test p>0.2). One variable, a single pre-foaling clostridial vaccination of the brood-mare, was eliminated because the vaccine was only given to mares on premises that had cases of *C. perfringens* and its application in an equine preventive medicine program was rare (USDA:APHIS:VS, 1998b). Thus, the use of this vaccine appeared to increase the risk of having a case foal, but the biologic plausibility of such a causal relationship seems doubtful. The use of this vaccine is more likely to be a marker for the occurrence of the disease on the premises. Almost half of the 42 variables (19/42) were not associated with the outcome of interest (p>0.2). These variables were also eliminated from further analysis.

The remaining 13 variables were deemed to be potential risk factors for enterocolitis associated with *C. perfringens* infection (p<0.2) and were included in the multivariable logistic-regression model. These variables are indicated in Table 1 with a superscript a.

From the multivariable logistic-regression model, six variables were significantly associated with an increased risk of the outcome (p<0.05): foals of the stock horse breeds, low amounts of grass hay (<6.8 kg) fed after foaling, low amounts of grain (<2.7 kg) fed

Table 2
Final multivariable logistic-regression model for risk factors associated with the occurrence of enterocolitis attributed to *C. perfringens* in foals ($p<0.05$) (Colorado, USA; 1996–1997)

Variable	Categories	Odds ratio	95% confidence interval
Breed type	Stock horse (Quarter Horse, Paint, and Appaloosa)	5	1.08–25
	Non-stock horse (all other breeds)	1.0	
Grain pre-partum (kg/day)	<2.7	0.04	0.01–0.26
	≥2.7	1.0	–
Grain post-partum (kg/day)	<2.7	63.63	7.8–520
	≥2.7	1.0	–
Grass hay post-partum to brood-mare (kg/day)	<6.8	5.62	1.28–25
	≥6.8	1.00	–
Housing type day 3 of life	Stall only	48.6	4.1–529
	Stall and outside access	9.1	1.11–79
	Drylot	22.8	1.3–398
	Pasture	1.0	–
Livestock on premises in past	Yes	11.1	2.5–50
	No	1.0	–

after foaling, housing in a stall or drylot in the first three days of life, and the presence of livestock other than horses on the premises in the past (Table 2). Low amounts of grain (<2.7 kg) fed prior to foaling was associated with a decreased risk of having an affected foal. None of the interaction terms remained in the model. The non-design based measure of goodness-of-fit for the model gave a Hosmer–Lemeshow goodness-of-fit statistic of 2.62 ($p=0.92$). Though this statistic does not take into account the clustered nature of the data, it does indicate a generally good agreement between the model and the data under an assumption of independence of observations.

4. Discussion

Stock-horse mares (Quarter Horse, Appaloosa, and Paint) were more likely to have affected foals than other mares. The relationship between brood-mare breed and the risk of being a case could be related to breed-associated differences in milk composition, amount of milk produced, or amount of milk ingested by foals. These factors may have led to altered fermentation in the gastrointestinal tract or variations in gastrointestinal susceptibility to overgrowth of this enteric pathogen (i.e. protease inhibitor levels, flora colonization, etc.). In addition, other unmeasured factors associated with the breed of mare/foal or management of these animals not measured in the current study could have been responsible for the observed association. Stock-horse breeds are often used to herd or work with other livestock (such as cattle) and are housed with or near these animals.

Though the presence of other livestock on the premises was controlled for in the analysis (and was significantly associated with the outcome), there may be other more-specific livestock-associated risk factors not assessed in this study that may contribute to the occurrence of this disease in stock-horse-breed foals.

The historical presence of other livestock on the premises was also associated with an increased risk of being a case. Other livestock may be subclinical shedders of clostridial organisms (thus increasing the environmental load of clostridial organisms). *C. perfringens* type A may be cultured from feces and intestines of many different domestic livestock species in the absence of clinical disease (Milev, 1976; McDonel, 1980; Hatheway, 1990; Songer, 1996). Cattle premises also routinely vaccinate cattle against *C. perfringens* type C and D (Clarkson et al., 1985; USDA:APHIS:VS, 1994) which may further contribute to subclinical shedding of these organisms by cattle (Michelson, 1996; Songer, 1996). A foal born in such an environment, to a naïve mare, may be at increased risk of developing clostridial enterocolitis. In a study of cattle carcasses, contaminated with *C. perfringens* that were buried on a premises, *C. perfringens* was more prevalent and more frequently cultured from the soil in the area surrounding the burial sites as well as from rain drainage from the burial sites in the spring of the second and third year than from the first year after burial of contaminated animals (Davies and Wray, 1996). This delayed and seasonal presence of *C. perfringens* in soil samples may shed some light on the reason that past versus current presence of other livestock on a premises represented an increased risk of disease. Our study did not investigate method or location of carcass disposal of livestock on the premises.

There is also evidence that the lethal *C. perfringens* type C is rarely found in the environment (unlike type A) (Hatheway, 1990; Songer, 1996). *Clostridium perfringens* type C is found predominantly on premises with an endemic livestock-based problem with this enteric pathogen (Hatheway, 1990; Songer, 1996). Thus, the historical presence of other livestock could serve as the nidus for setting-up an endemic disease that may affect susceptible foals. There may be a risk of *C. perfringens* contamination when building a new horse facility on a premises previously occupied by other livestock.

Foals housed in stalls without outside access and foals housed on drylots were at increased risk compared to foals housed on pasture or in stalls with outside access. Foals in a pasture environment may have a diluted exposure relative to a foal kept confined which may suckle on the floor, wall, feeders, etc. in its first hours of life. There is also an increased potential contact with the mare's feces and exposure to clostridial organisms or spores if present in the mare's feces. Foals maintained in a drylot were also at increased risk over pasture kept foals for the first three days of life. Because *C. perfringens* is present in the environment, a barren dirt paddock may increase the potential for a naïve foal to ingest dirt laden with clostridial spores and develop disease as opposed to a foal born in a grass pasture (Hatheway, 1990; Songer, 1996). Heavy manure build up in stalls or paddocks may further augment risk of spore exposure (Milev, 1976). We did not investigate methods of manure handling or disposal on premises in our survey.

Three nutrition-related variables were associated with the occurrence of clostridial associated enterocolitis. Low levels of grain (<2.7 kg/day) feeding prior to foaling was associated with a decreased risk of being a case while low levels of grain (<2.7 kg/day) and low levels of grass hay (<6.8 kg/day) after foaling were associated with an increased risk of being a case. Low levels of grain prior to foaling may have affected brood-mare body condition and subsequent milk production. The increased risk of being a case if the mare was fed low amounts of grain after foaling is the opposite of the expected finding based on extrapolation of information from other species and current knowledge of the pathogenesis of this disease. There does not appear to be a straightforward explanation for this finding. However, characterizing the ration as 'low' in grain and 'low' in grass hay means that other nutrients must have been fed to these animals. Perhaps the combination of the other nutrients such as alfalfa hay and pasture were responsible for increasing the risk of having a case foal. This study did not quantify the amount of pasture consumed or quality of the pasture, hay, or grain source. In addition, owners were asked to estimate the amounts of each feed ingredient fed. Perhaps owners with case foals underestimated the amount of grain fed because of subsequent knowledge about the disease and expected pathogenesis (differential misclassification). The negative association of high grain feeding is also difficult to explain since presumably these animals did receive some form of forage (another hay source or pasture) which might be expected to be higher in energy than grass hay. Unfortunately, the data currently available from this study did not allow us to fully evaluate these nutrition related hypotheses, since further follow-up with responding premises was not possible based on imposed anonymity of respondents. Additionally, if a lower nutritional plane or energy ration existed, it could be an indicator of an overall lower level of animal care on the premises.

Because of the unexpected nature of the associations of the nutrition variables with the outcome and to see what the impact of the nutrition variables had on the other variables offered to the model, we constructed another model excluding the nutrition variables from consideration. Three variables consistently remained in the new model: stock-horse breed (OR=10.0), livestock other than horses present in the past (OR=9.1), and housing location in the first three days of life (stall OR=47.5, stall with pasture OR=6.2, drylot OR=43.4) and the magnitude of the associations were essentially unchanged from the model containing the nutrition variables. One new variable did remain in the model: the type of ground/floor surface in the foaling facility. Ground/floor surface that was difficult to clean (dirt, sand, or gravel) was associated with an increased risk (OR=14.3, 95% CI 1.3, 152.8) of being a case compared to foaling facilities with ground/floor surface of cement, rubber, or wood. One may speculate that differences in the pH, moisture level, and other factors may impact the role different ground/floor surface types play as risk factors in this disease. It is encouraging that regardless of whether the nutritional associations are spurious or real, the remaining environmental and animal factors seem to be independently associated with the outcome of interest.

The efficacy of several previously proposed interventions could not be demonstrated in this study (Fukata et al., 1991; Traub-Dargatz and Jones, 1993; East et al., 1998). Although pre-partum vaccination of the brood-mare with *C. perfringens* type C and D toxoid could not be analyzed thoroughly in these data, none of the brood-mare's vaccinated twice pre-partum with *C. perfringens* type C and D toxoid (n=11) had affected foals, while 7 of 12 mare's vaccinated once had affected foals. If vaccination of brood-mare's with *C. perfringens* type C and D toxoid is a potential marker for the disease on a premises, then the initial data suggest that vaccination of the mare's twice pre-partum versus once might impart colostral immunity to foals on affected premises. Some field veterinarians and equine owners have reported to one of the authors (LME) the occurrence of severe injection-site reactions in horses with the extra-label application of oil-based adjuvant formulations of *C. perfringens* type C and D toxoid. Other recommended practices including oral *Lactobacillus acidophilus*, or *C. perfringens* type C and D anti-toxin, and administration of intramuscular penicillin to neonatal foals (Fukata et al., 1991; Traub-Dargatz and Jones, 1993; East et al., 1998) could not be evaluated in this study due to infrequent use in the study population.

Inherent in most retrospective studies as in ours, there are some biases that may impact data interpretation. Response bias may have occurred. Proportionately fewer of the control premises (14/37) reported foal data for both 1996 and 1997, despite noting that foals were born in 1996 on the questionnaire. In 1996, more premises with affected foals might have responded due to an increased proportion of these premises being willing to provide data for both years. Indeed nine of the 17 premises with case foals reported on foalings in both years. Though the response rate for the survey was only 46%, this represents a substantial return to a mailed-out questionnaire with limited follow-up contact. Still, with non-response comes the potential for bias in the data and thus conclusions should be made with caution and further research should be done to confirm the findings. As for bias attributed to survey-population selection, we consider the selected population a reasonable representation of the Colorado population in that both case and control farms were selected from this population.

This study demonstrated a high case-mortality risk for this disease, 68%, versus a mortality risk of 4% for the remainder of the population. This case-mortality risk coincides with previous reports of a high case-mortality risk, 54%, with this disease (East et al., 1998). The mortality risk for foals of any cause in previous reports was between 3.6 (USDA:APHIS:VS, 1998a) and 4.7% (Cohen, 1994). This mortality risk is very close to that of the control population in our study. This low mortality risk for the general foal population further underscores the markedly increased case-mortality risk associated with this disease.

Some avenues of future research that we suggest include environmental and livestock fecal cultures for *C. perfringens* type C and type A to document its prevalence regionally. A more in-depth climate and moisture evaluation might also elucidate the previously documented spring rise in positive *C. perfringens* environmental soil cultures on contaminated premises (Davies and Wray, 1996). Structured intervention trials on premises with affected foals might also aid in evaluating efficacy of prevention methods.

5. Conclusion

This study provided information on some of the general foaling practices of premises in Colorado, USA and elucidated potential risk factors for enterocolitis attributed to *C. perfringens* infection in the equine neonate. Environmental and management factors that might be associated with increased risk for equine neonatal clostridial enterocolitis include: foals born to stock-horse brood-mares, periparturient housing in a stall or drylot, ground/floor surface types in the foaling area such as dirt, sand, or gravel, and the historical presence of livestock other than horses on a premises. Brood-mare ration recommendations to aid in the prevention of *C. perfringens* enterocolitis in the neonate are not readily gleaned from our survey and require further investigation to determine their significance.

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